云 南 植 物 研 究 1996; **18** (1): 110～114
Acta Botanica Yunnanica

翅萼龙胆中的两个寡聚裂环烯醚萜甙

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摘要 从翅萼龙胆（*Gentiana kusnezowii*）的全株中分离得到两个新的寡聚裂环烯醚萜甙：翅萼龙胆甙（kusnezoside）Ia 和 Ib，其结构通过光谱分析推定。

关键词 翅萼龙胆，龙胆科，寡聚裂环烯醚萜甙，翅萼龙胆甙 Ia 和 Ib

TWO NEW OLIGOSECOIRIDOID GLYCOSIDES FROM GENTIANA KUSNEZOWII

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Abstract Two new oligosecoiridoid glycosides named kusnezosides Ia and Ib were isolated from the whole plant of *Gentiana kusnezowii.* Their structures were determined by spectroscopic analysis.

Key words *Gentiana kusnezowii,* Gentianaceae, Oligosecoiridoid glycosides, Kusnezosides Ia and Ib

Gentiana kusnezowii Franch. is a gentianaceous herb endemic in the middle and southern area of Yunnan province of China. It is used in Wenshan county by the Zhuang people for the treatment of pneumonia, bronchitis, hepatitis and cholecystitis. In a continuation of chemical studies of the genus *Gentiana* in our research group, we have now investigated the whole plant of *G. kusnezowii,* which led to the isolation of two new oligosecoiridoid glycosides, kusnezosides Ia (**1**) and Ib (**2**).

Kusnezoside Ia (**1**) was obtained as a white powder. The ^{13}C and ^{1}**H NMR** spectra are quite similar to those of dimethyl ester of rhodenthoside A (**3**), a new acylated secoiridoid glycoside from *Gentiana rhodentha* (Ma *et al,* 1994) which was composed of a monoterpene dicarboxylic acid and two secologanol methyl ester units (Tables 1 and 2). However, compound **1** showed in the ^{13}**C NMR** spectrum additional signals in the carbonyl field region of δ 168.6～169.4 and hydroxymethyl field region of δ 63.5～64.4. In addition, the integral intensities of some proton signals of secologanol units are doubled compared to compound 3(Table 2). Combined with the **FAB**-mass spectrum which displayed a quasi-molecular ion peak at m/z 1659[M+H]$^-$, the molecular formula of **1** was determined as $C_{76}H_{106}O_{40}$. This indicated compound **1** was formed from **3** by increasing two secologanol units. As the monoterpenic acid is not symmetrical, the sequence of secologanol methyl ester (part a), secologanol (part b) and monoterpene dicarboxylic

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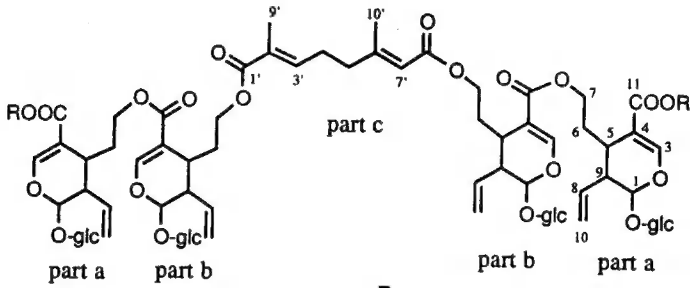
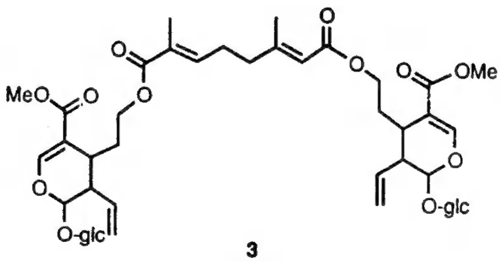
1995-09-04 收稿，1995-11-04 修回

acid (part c) in **1** has three possibilities: i) a—b—c—a, ii) a—b—b—c—a, and iii) a—c—b—b—a. It is difficult for **NMR** techniques to resolve this problem. In some cases, **FAB–MS** have a great advantage to determine molecular structures. In the case of **1** , it has to be pointed out that the **FAB–MS** analysis can not differentiate the latter two sequences, because they give rise to same fragmentation patterns. In the negative **FAB–MS** of **1**(Fig.1), fragment ion peaks appeared at *m/z* 1496[M−Glc]$^-$, 1286[M−a($C_{17}H_{24}O_9$)]$^-$, 928[1286−b($C_{16}H_{22}O_9$]$^-$, 749[928−c]($C_{10}H_{12}O_3$)+H]$^-$, 555 [928−a($C_{17}H_{24}O_9$)]$^-$and 197 [c ($C_{10}H_{12}O_4$)+H]$^-$. In addition, any characteristic ion peaks derived from a terminal secologanol methyl ester unit linked with the monoterpene moiety [a—c] or a terminal secologanol methyl ester unit linked with two secologanol units [a—b—b] were not observed, indicating that the latter two possibilities could be excluded. The $^1H-^1H$ COSY, $_1H-^{13}C$ COSY and COLOC experiments were performed and most proton and carbon signals were assigned. Based on the above evidence, the structure of **1** was established.



	R
1	Me
2	H



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Kusnezoside Ib (**2**) was obtained as a yellow powder. A quasi-molecular ion peak at *m/z* 1631 [M+H]$^-$ in the FAB–mass spectrum suggested that its molecular formula should be $C_{74}H_{102}O_{40}$. The ^1H and $_{13}C$ NMR spectra of **2** revealed that it was a derivative of **1**. Compound **2** showed a singlet peak at δ 7.25 (2H, H−3 × 2) and its corresponding carbon signal at δ 150.6, as well as a carbon signal at δ 114.5 assignable to C−4 of secologanol unit. Furthermore, no carbon and proton signals of methoxyl groups appeared. This indicated that two of C−11 carboxylic groups were free. Thus, compound **2** was the demethyl product of **1**.

It is known that the methyl group attached to C–11 in iridoid glycosides is biogenetically synthesized in plants. In this study, we isolated the carboxylic free form (2) and its methyl ester (1). We have not yet confirmed which one is an artefact.

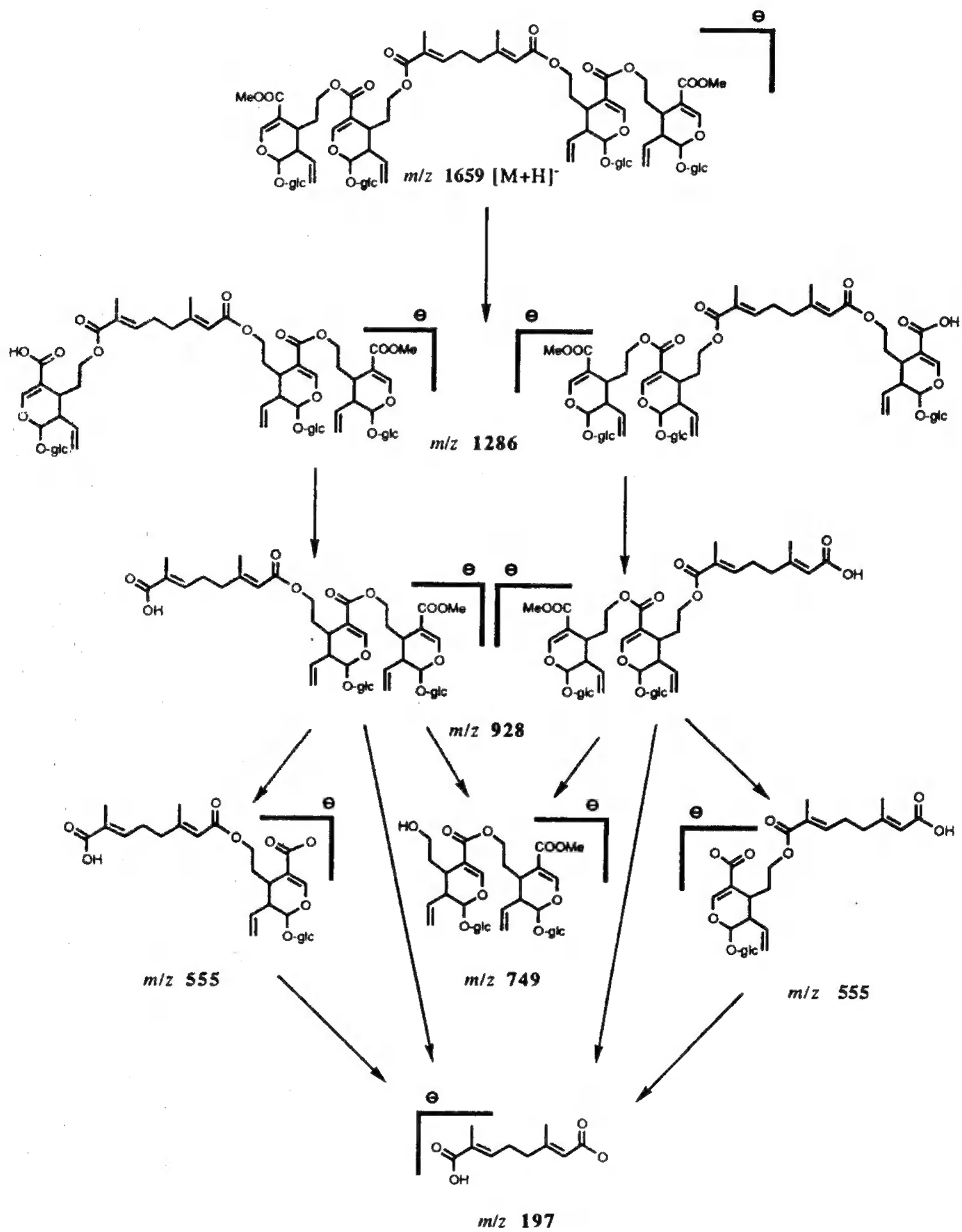

Fig.1 Negative FAB-MS fragmentation pattern of **1**

Table 1 ^{13}C-NMR spectral data of **1**–**3**in CD_3OD (δ, ppm)

	2			**1**			**3***	
Aglycone	part b	part b	part a×2	part b	part b	part a×2	part b	part a
C-1[a)]	97.5	97.5	97.8	97.8	97.8	97.8	97.4	97.5
C-3	153.7	153.7	150.6	153.6	153.6	153.6	153.2	153.1
C-4	111.7	111.7	114.5	111.7	111.7	111.4	111.3	111.3
C-5[b)]	31.2	31.2	31.4	31.4	31.4	31.4	31.2	31.3
C-6[c)]	29.8	29.8	30.0	30.0	30.0	30.0	29.9	29.8
C-7[d)]	64.4	63.5	63.7	64.4	63.5	63.7	63.9	63.1
C-8[e)]	135.8	135.8	135.5	135.6	135.6	135.6	135.3	135.2
C-9	45.2	45.2	45.2	45.2	45.2	45.2	27.9	27.9
C-10[f)]	119.8	119.8	119.6	119.7	119.7	119.7	119.1	119.1
C-11[g)]	168.7	168.7	169.5	168.6	168.6	169.1	167.7	167.7
OMe						51.9	51.3	51.2
Glucosyl								
C-1		100.2,	100.0		100.3		99.9	
C-2		74.6			74.6		74.4	
C-3		78.0			78.0		77.7	
C-4		71.6			71.6		71.3	
C-5		78.3			78.3		78.0	
C-6		62.8			62.8		62.5	
Monoterpene(part c)								
C-1'		169.5			169.4		169.1	
C-2'		129.6			129.4		129.3	
C-3'		142.3			142.2		141.7	
C-4'		27.6			27.6		27.1	
C-5'		40.2			40.3		39.9	
C-6'		160.2			160.2		159.6	
C-7'		117.0			117.1		116.8	
C-8'		168.1			168.1		168.8	
C-9'		12.7			12.7		12.1	
C-10'		19.1			19.1		18.5	

* Cited from ref. (Ma et al, 1994). The values of C-9 are printing mistakes. They should be about δ 45.

a–g) Signals in each row for each compound may be interchangeable.

EXPERIMENTAL

Optical rotation were on a J-20C polarimeter. ^{13}C NMR and ^{1}H NMR spectra were measured on a Brucker spectrospin AM-400 spectrometer using TMS as aninternal standard. FAB-MS spectra were measured with VG AutoSpect mass spectrometer. CC was carried out with silica gel H, Sephadex LH-20, and Lichroprep RP-8, RP-18 (40–63μ, Merk), and TLC was conducted on a precoated Kieselgsel 60 F_{254} plate (0.2 mm, Merk) sprayed with 10% H_2SO_4 followed by heating.

Extraction and isolation Fresh whole plants (504g) of *Gentiana kusnezowii* during its early blossom period were collected in Wenshan county, Yunnan Province, P. R. China and was soaked in MeOH for four times. Removal of the solvent *in vacuo* afforded a black syrup (11g). This was suspended in 200 ml water and defatted by petroleum and ether. The water layer was chromatographied on MCI gel eluting with H_2O,

30% MeOH, 50% MeOH, 70% MeOH, 90% MeOH and MeOH to yield six fractions. Fr. 5 was repeatedly chromatographied on RP-18 (40% MeOH) and RP-8 (45% MeOH) to yield **2** (45 mg). Fr. 6 was repeatedly chromatographied on RP-18 (60% MeOH) and RP-8 (55% MeOH to furnish **1** (215 mg). The purity of compound **1** was checked with HPLC using ODS column, 55% MeOH at UV 230 nm.

Table 2 ^{1}H-NMR spectral data of **1-3** in CD_3OD (δ, ppm)

	2	1	3*
Aglycone			
H-1	5.52(4H, m)	5.52(4H, m)	5.54(2H, m)
H-3	7.50, 7.48(1H each,s)	7.48, 7.47(1H each, s)	7.46(2H, s)
	7.25(2H, s)	7.46(2H,s)	
H-5	2.89 (4H, m)	2.88 (4H, m)	2.90 (2H, m)
Ha-6	2.07 (4H, m)	2.04 (4H, m)	2.35 (2H, m)
Hb-6	1.76 (4H, m)	1.78 (4H, m)	1.95 (2H, m)
H-7	4.15 (8H, m)	4.15 (8H, m)	4.12 (4H, m)
H-8	5.76 (4H, m)	5.76 (4H, m)	5.79 (2H, m)
H-9	2.65 (4H, m)	2.66 (4H, m)	2.65 (2H, m)
Ha-10	5.25 (8H, m)	5.25(8H,m)	5.31(2H,d,J=14.0)
Ha-10			5.24(2H,d,J=10Hz)
OMe		3.67(6H,s)	3.65(6H,s)
Monoterpene (part c)			
H-3'	6.71 (1H, dd, J=8.2,4,1)	6.72 (1H, dd, J=8.2,4.1)	6.71 (1H, t, J=8.2,4.1)
H-4'	2.40 (2H, m)	2.40 (2H, m)	2.40 (2H, m)
H-5'	2.32 (2H, m)	2.32 (2H, m)	2.35 (2H, m)
H-7'	5.69 (1H, brs)	5,68 (1H, brs)	5.69 (1H, brs)
Me-9'	1.83 (3H, s)	1.83 (3H, s)	1.85 (3H, brs)
Me-10'	2.16 (3H, s)	2.16 (3H, s)	2.17 (3H, brs)
Glc H-1	4.72 (4H, m)	4.72 (4H, m)	4.67 (2H, d)

* Cited from ref. (Ma et al, 1994)

Kusnezoside Ia (1) White powder. $[\alpha]_D^{25}$-125.2° (MeOH, C=0.56). FAB-MS (negative): Fig.1. ^{1}H and ^{13}C NMR: Tables 1& 2.

Kusnezoside Ib (2) Powder. FAB-MS (negative)*m/z:* 1631 $[M+H]^-$, 1272 $[M-b\ (C_{16}H_{22}O_9)]^-$, 914 $[M-2b\ (C_{16}H_{22}O_9)]^-$, 555$[(b+c)\ (C_{26}H_{35}O_{13})]^-$, 197 $[c\ (C_{10}H_{13}O_4)]^-$. ^{1}H and ^{13}C NMR: Tables 1& 2.

Acknowlegements The authors are grateful to the analytical group of the Laboratory of Phytochemistry of our Institute for performing NMR and FAB-MS spectra.

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